AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning on page 1, line 12 as follows:

Fas is a cell surface antigen which transmits an apoptosis signal to the cell, and Fas is recognized by Fas antibody (Yonehara, S. et al., J. Exp. Med. vol. 169, 1747-1756, 1989), which is a monoclonal antibody produced by immunizing a mouse with human fibroblast. Fas gene was recently cloned by Itoh, N. et al., and it was then found out that Fas is a cell membrane protein of about 45 kD, and from the amino acid sequence, it was revealed that Fas is a member of the TNF receptor family (Cell, vol. 66, pages 233 - 243, 1991). ~~Mouse~~ The mouse Fas gene was also cloned (Watanabe-Fukunaga, R. et al., J. Immunol., vol. 148, pages 1274 - 1279, 1992), and its expression in thymus, liver, lung, heart, ovary was confirmed.

Please amend the paragraph beginning on page 1, line 24 as follows:

Human Fas ligand is a polypeptide which has been reported by Nagata et al. to be a biological molecule which induces apoptosis of Fas-expressing cells (Tomohiro Takahashi et al., International Immunology, vol. 6, pages 1567 - 1574, 1994). Human Fas ligand is a Type II membrane protein of the TNF family with a molecular weight of about 40 kD. As in the case of TNF, human Fas ligand in the human body is estimated to be in the form of a trimer (Masato

Tanaka et al., EMBO Journal, vol. 14, pages 1129 - 1135, 1995). The extracellular domain of ~~the~~ human Fas ligand is highly homologous with the extracellular domain of rat Fas ligand (Takashi Suda et al., Cell, vol. 75, pages 1169 - 1178, 1993) and mouse Fas ligand (Tomohiro Takahashi et al., Cell, vol. 76, pages 969 - 976, 1994). ~~The human~~ Human Fas ligand recognizes not only ~~the~~ human Fas but also ~~the~~ mouse Fas to induce ~~the~~ apoptosis~~,~~; and vice versa, ~~the~~ rat Fas ligand and ~~the~~ mouse Fas ligand also recognize ~~the~~ human Fas to induce ~~the~~ apoptosis.

Please amend the paragraph beginning on page 2, line 14 as follows:

Considerable researches have also been done on the mechanism of signal transduction in the cell upon ~~the~~ Fas-mediated apoptosis, and identification and cloning of the factor which interacts with the intracellular domain of ~~the~~ Fas, in particular, the region called "death domain" to transmit or block the signal ~~have~~ has been reported. Possibility of the involvement of interleukin-1-converting enzyme (ICE)-related thiol proteases in ~~the~~ Fas-mediated apoptosis has also been indicated.

Please amend the paragraph beginning on page 2, line 23 as follows:

A relationship ~~Relationship~~ between ~~the~~ apoptosis, in particular, ~~the~~ Fas-mediated apoptosis with various diseases and

physiological phenomena has been recently indicated. For example, possibility has been indicated for involvement of abnormal Fas-mediated apoptosis in the decline of the T cell count in ~~the~~ patients suffering from AIDS, in the death of hepatocytes in viral fulminant hepatitis, in some types of autoimmune diseases, and the like.

Please amend the paragraph beginning on page 3, line 5 as follows:

Involvement of the Fas/Fas ligand system ~~is~~ in functions other than ~~the~~ apoptosis has also been indicated. For example, the possibility has been indicated for the Fas/Fas ligand system to react with neutrophils to develop a proinflammatory action (Kayagaki, N. et al., Rinshou Meneki (Clinical Immunology), vol. 28, pages 667 - 675, 1996).

Please amend the paragraph beginning on page 3, line 11 as follows:

The condition wherein neutrophils and other immunocompetent cells are activated by various ~~stimulation~~ stimuli, such as ~~endotoxin~~ endotoxins or by invasion, and humoral factors, such as ~~cytokine~~ cytokines, are released into the blood and the tissues to induce systemic inflammatory responses is referred as systemic inflammatory response syndrome (hereinafter abbreviated as SIRS). SIRS is often associated with various organ failures, and when such

organ failures are serious, SIRS may result in multiple organ failure syndrome (MODS) (Wakabayashi et al., Rinsho Kensa (Laboratory test), vol. 38, pages 349 - 352, 1994). Various factors are said to be involved in the process of ~~the~~ organ failure, and the role in such organ failure of the invasion and accumulation of ~~the~~ neutrophils by the action of IL-8 produced in macrophages and of other cells by IL-1 locally produced in response to invasion is of current interest. As described above, the Fas/Fas ligand system (hereinafter referred to as Fas/FasL system) is also reported for the possibility of its involvement in the activation of the neutrophils.

Please amend the paragraph beginning on page 4, line 3 as follows:

Ischemic reperfusion injury is found in practically all tissues and organs, and is involved in various diseases. Ischemic reperfusion injury is also a problem in preservation and transplantation of organs. Among such ischemic reperfusion injuries, those associated with infarction of liver, heart or kidney and those associated with surgery or transplantation, and in particular, tissue injury (such as cell necrosis) and dysfunction (such as cardiac arrhythmia) in the particular organ may lead to the death of the individual when they are serious, and therefore, such cases are a serious social problem. Various organ failures

and ischemic reperfusion injuries ~~of~~ from the early stage to the late stage are known to be associated with production and secretion of IL-8. It is also known that organ preservation and reperfusion in the course of organ transplantation is associated with the occurrence of the apoptosis. In addition, observation of apoptosis and fluctuation in the expression of Fas or FasL have been reported for some experimental models. There have also been reported a marked increase in the number of neutrophils at 24 hours after the reperfusion of liver after ischemia, and improvement of ischemic reperfusion injury by ~~the~~ a neutralizing antibody of ~~the~~ neutrophils (Jaeschke, H. et al., FASEB Journal, vol. 4, pages 3355 - 3359, 1990), and a marked increase of IL-8, neutrophils and macrophages at 3 hours after the reperfusion of the lung after ischemia, and improvement of ischemic reperfusion injury by ~~the~~ a neutralizing antibody of the IL-8 (Sekido, N. et al., Nature, vol. 365, pages 654 - 657, 1993). These findings suggest ~~the~~ significant roles of ~~the~~ neutrophils and ~~the~~ IL-8 in organ failures and ischemic reperfusion injuries of from the early stage to the late stage. On the other hand, it is not yet found how ~~the~~ Fas/FasL is involved in such failures and injuries.

Please amend the paragraph beginning on page 5, line 8 as follows:

In ~~the~~ infection by bacteria, endotoxin induces production of various cytokines in the body resulting in, for example, endotoxin shock in endotoxemia and sepsis as well as various organ damages including ~~the~~ liver damage (Dinarello, C.A. et al., J. American Medical Association, vol. 269, page 1829, 1993), and serious conditions are more than often induced. Observation of ~~the~~ apoptosis in such ~~process~~ processes and the possibility of some involvement in such ~~process~~ processes of ~~the~~ Fas/FasL have been reported in experimental studies. However, it is not yet found how ~~the~~ Fas/FasL is involved in such failures.

Please amend the paragraph beginning on page 5, line 18 as follows:

Death of cardiomyocytes in the case of a heart disease has been believed to occur mainly through necrosis. However, possibility of some involvement of ~~the~~ apoptosis, and in particular, involvement of ~~the~~ Fas-mediated apoptosis in such heart disease is now reported for clinical practices and in experiments. For example, ~~there have~~ it has been reported that the level of Fas expression increases when neonatal rat cardiomyocytes are placed in ~~an~~ in vitro ischemic conditions (Tanaka, M. et al., Circ. Res., 75, 426 - 433, 1994); and that NO may have some relation to the remodeling of plaques in arteriosclerosis since IL-1 induces not

only the synthesis of nitrogen monoxide (NO) of vascular smooth muscle cells but also apoptosis, apoptosis by IL-1 is inhibited by an inhibitor of ~~the~~ NO synthesis, and Fas expression is induced by NO (Fukuo, K. et al., Hypertension, 27, 823 - 826, 1996). ~~There have~~ It has also been reported that apoptosis of cardiomyocytes is found in a canine heart failure model and such apoptosis is associated with an increased Fas expression (Lab. Invest., 73, 771 - 787, 1995), and that most of the cardiomyocyte death in canine the myocardial infarction model occurs through apoptosis, and such cardiomyocyte death is associated with a ~~100 fold~~ 100-fold increase in ~~the~~ Fas expression (Lab. Invest., 74, 86 - 107, 1996). Furthermore, Fukuda et al. examined Fas expression in cardiomyocytes of cardiomyocardial disease patients, and found no Fas expression in hypertrophic cardiomyopathy but some Fas expression in at least a part of cardiomyocytes in ~~the~~ cases of myocarditis and dilated cardiomyopathy (Idiopathic Cardiomyopathy Investigation Group, 1994 Business Year Research Report, 152 - 155, 1995). However, it is yet to be found out how ~~the~~ Fas is involved in such heart diseases. Accordingly, the reports as described above ~~has~~ have provided no data with regard to whether ~~the~~ Fas acts to promote the apoptosis of the cardiomyocytes or to suppress the apoptosis of the cardiomyocytes in heart diseases, and it was still unclear whether ~~the~~ Fas is directly involved in cytotoxicity or

death of the cardiomyocytes of the patients suffering from heart diseases. As a consequence, no therapeutic agent and no therapy for ~~the~~ heart disease wherein the disease, is treated by inhibiting the Fas-mediated apoptosis ~~are~~ is known to date.

Please amend the paragraph beginning on page 7, line 10 as follows:

Graft versus host disease (hereinafter referred to as GVHD) is a disease caused by graft versus host reaction (GVH reaction), which is an immunoreaction that may occur upon transplantation of ~~the~~ lymphocytes of ~~the~~ a donor or ~~the~~ a graft, against the tissue ~~antigen~~ antigens of the host. Exemplary GVHDs are GVHD after bone marrow transplantation, such as ~~the~~ with incompatible bone marrow transplantation or ~~the~~ with bone marrow transplantation in congenital immune deficiency syndrome; GVHD after organ transplantation; GVHD after blood transfusion, in which a large amount of blood is transfused to a patient of hypoimmunity; and the like. GVHD is associated with organ or tissue failure based on GVH reaction, and diarrhea, exhaustion such as weight loss and thinning, exanthem, splenomegaly, and liver dysfunction are clinically observed. GVHD is also associated with histological symptoms such as disorganization of bone marrow and lymphoid tissue and atrophy of intestinal villi.

Please amend the paragraph beginning on page 7, line 26 as follows:

Death of the cells constituting the host tissue in various GVHD has been believed to occur mainly through necrosis. However, the possibility of some involvement of ~~the~~ apoptosis, and in particular, ~~the~~ Fas-mediated apoptosis in such GVHD is now reported ~~for~~ from experiments. For example, ~~there~~ it has been reported that death of epithelial cells in intestine, skin and tongue in a mouse GVHD model occurs mainly through apoptosis (Aniti C. Gilliam et al., J. Invest. Dermatol., vol. 107, pages 377 - 383, 1996). With regard to the involvement of ~~the~~ Fas-mediated apoptosis, ~~there~~ it has been reported that no difference was found in survival time between ~~the~~ cases when the donor was spleen lymphocytes from a control mouse with normal Fas ligand and ~~the~~ cases when the donor was spleen lymphocytes from a gld mouse, which is a Fas ligand-mutated mouse, and practically no damage in skin and liver was induced (Matthew, B., Barker, B. et al., J. Exp. Med., vol. 183, 2645 - 2659, 1996). In spite of the indication of the involvement of ~~the~~ Fas-mediated apoptosis in ~~the~~ GVHD, there is no conclusion with regard to whether the Fas-mediated apoptosis is related to ~~the~~ mortality ~~of~~ with the GVHD. Furthermore, the report as described above utilizes spleen lymphocytes from a gld mouse for the material, and it is likely that the GVHD reaction is influenced by alteration in the amount of the expression of ~~the~~ factors other

than ~~the~~ Fas ligand (such as perforin and TNF) as a substitute for the lack of ~~the~~ Fas ligand, and the results obtained may not necessarily reflect the genuine effect of the lack of ~~the~~ Fas ligand. Therefore, it is yet to be found out how ~~the~~ Fas-mediated apoptosis is involved in GVHD, and whether ~~the~~ a substance which specifically inhibits ~~the~~ Fas-mediated apoptosis can be used as a therapeutic agent for ~~the~~ GVHD.

Please amend the paragraph beginning on page 9, line 4 as follows:

~~The nonspecific~~ Nonspecific immunosuppressives, such as cyclosporin, that have been used as a prophylactic or therapeutic agent of ~~the~~ GVHD ~~generates~~ generate nonspecific immunosuppression, and therefore, suffer from adverse side effects such as infections. No therapeutic agent and no therapy for ~~the~~ GVHD wherein the GVHD is treated by inhibiting ~~the~~ Fas-mediated apoptosis are known to date. In addition, no therapeutic agent and no therapy for ~~the~~ GVHD wherein the GVHD is treated by utilizing selective immunosuppression are known to date.

Please amend the paragraph beginning on page 9, line 13 as follows:

With regard to ~~the~~ diseases based on ~~the~~ ischemic reperfusion injury, commercially available drugs mainly ~~aims~~ aim at thrombolysis and improvement of circulation, and no drug is

available that directly prevents or treats the damage. With regard to the endotoxemia and sepsis, steroid and proteolytic enzyme inhibitor, for example, are used in the case of shock, and no drug is currently available that directly prevents or treats the organ damage. The drugs used for the diseases based on organ damage mainly ~~aims~~ aim at palliative treatment, and no drug is available that prevents or radically treats the diseases based on organ damage. In addition, no prophylactic or therapeutic agent which ~~are~~ is widely effective for various tissues and organs ~~are~~ is available.

Please amend the paragraph beginning on page 9, line 26 as follows:

In view of such situation, there is a demand for a pharmaceutical which is effective in preventing or treating ~~the~~ diseases based on damage of the tissue or the organ in a wide ~~varieties~~ variety of tissues or organs, which is effective in vivo, and which is less toxic to ~~human~~ humans. However, no pharmaceutical is so far available that meets such requirements.

Please amend the paragraph beginning on page 10, line 16 as follows:

The inventors of the present invention have conducted an intensive study on function of the Fas/Fas ligand system and the

role of ~~the~~ apoptosis mediated by the Fas/Fas ligand system in various diseases, and found that conditions may be improved in various disease models by suppressing the actions of the Fas/Fas ligand system, and in particular, by suppressing the Fas/Fas ligand system-mediated apoptosis; and that, for example, that death of cardiomyocytes upon reperfusion after ischemia, onset of the GVHD associated with allogenic bone marrow transplantation, and organ damages caused by endotoxin are suppressed by an antagonist which inhibits the Fas-mediated apoptosis. The present invention has been completed on the bases of such finding.

Please amend the paragraph beginning on page 39, line 1 as follows:

After precipitating 1 liter of the culture supernatant of COS-1/pM1304 with ammonium sulfate (70% saturation), the precipitate was suspended in phosphate buffer saline (PBS) and dialyzed against PBS. To ~~Affi-Prep~~ AFFI PREP Protein A Preparative Cartridge Column (Protein A affinity chromatography matrix, manufactured by Biorad Inc.; 7.3 ml) was applied 57 ml of the resulting suspension, and the shFas(nd29)-Fc eluted was collected, and concentrated by ultrafiltration using ~~Filtron Omega Cell~~ FILTRON OMEGA CELL (polyetheusulfone ultracentrifugation membrane, manufactured by Fuji Filter K.K.; fractionating molecular weight: 30 kD). The concentrate was dialyzed against 0.9% NaCl to obtain the purified

shFas(nd29)-Fc. hFas-Fc was purified in a similar manner. The amount of the protein in each specimen was measured by Lowry's method using bovine serum albumin for the standard.

Please amend the paragraph beginning on page 39, line 14 as follows:

The resulting purified shFas(nd29)-Fc was electrophoresed on a 5 to 20% gradient polyacrylamide gel containing 0.1% SDS, and stained with ~~2D-Silver Stain-II~~ 2D-SILVER STAIN-II "Daiichi" (silver staining kit manufactured by Daiichi Kagakuyakuhin K.K.) to detect the bands. The purified shFas(nd29)-Fc was detected under non-reduced conditions as a substantially single band of about 85 kD corresponding to the dimer, and under reduced conditions as a substantially single band of about 43 kD corresponding to the monomer.

Please amend the paragraph beginning on page 39, line 23 as follows:

The resulting shFas(nd29)-Fc was applied to VYDAC C4 column (4.6 mm diam. x 25 cm, synthetic-silica-based reversed-phase chromatography column manufactured by Cypress Inc.) preliminarily equilibrated with 0.05% trifluoroacetic acid, and the column was washed with 0.05% trifluoroacetic acid. After the washing, the

column was eluted at linear concentration gradient method at a flow rate of 1 ml/minute using 0.05% trifluoroacetic acid/0 to 100% acetnitrile.

Please amend the paragraph beginning on page 40, line 25 as follows:

10 liters of the culture supernatant of COS-1/pM1317 was concentrated to 1.5 liters by ultrafiltration using ~~Filtron Mini~~ FILTRON MINI kit (fractionating molecular weight: 10 kD; manufactured by Fuji Filter K.K.). The concentrate was supplemented with 1M Tris-HCl (pH 9.0) to adjust the pH to 8.0, and the solution was applied to an affinity column having the anti-Fas antigen monoclonal antibody immobilized thereto which had been equilibrated with 50 mM Tris-HCl (pH 8.0) containing 1M NaCl. The column was washed with 320 ml of 50 mM Tris-HCl (pH 8.0) containing 1M NaCl, and shFas(nd29)-hinge was eluted with 0.1M glycine-HCl (pH 2.5). The fractions containing the shFas(nd29)-hinge were pooled, and concentrated by using ~~Filtron Omega Cell~~ FILTRON OMEGA CELL (fractionating molecular weight: 10 kD; manufactured by Fuji Filter K.K.). The concentrate was dialyzed against 0.9% NaCl to obtain the purified shFas(nd29)-hinge.

Please amend the paragraph beginning on page 41, line 15 as follows:

The resulting purified shFas(nd29)-hinge was electrophoresed on a 5 to 20% gradient polyacrylamide gel containing 0.1% SDS, and stained with ~~2D-Silver Stain-II~~ 2D-SILVER STAIN-II "Daiichi" (manufactured by Daiichi Kagakuyakuhin K.K.) to detect the bands. The purified shFas(nd29)-Fc was detected under non-reduced conditions as two bands at a molecular weight of about 43 kD and about 27 kD, and under reduced conditions as two bands of about 23 kD and 27 kD.

Please amend the paragraph beginning on page 46, line 24 as follows:

The value of creatine kinase (CPK) in plasma was measured by using CPK ~~TestWako~~ TESTWAKO and autoanalyzer (COBAS FARA, manufactured by Roche).

Please amend the paragraph beginning on page 49, line 16 as follows:

16 µg of plasmid pM1304 was dissolved in 5.5 µl of 10mM Tris-HCl (pH 7.4)/1mM ethylenediaminetetraacetic acid solution. F-12 Nutrient Mixture (Ham) medium was added to this solution (3:1 (w/w) liposome formulation of DOSPA and DOPE, manufactured by GIBCO BRL

Inc., hereinafter abbreviated as HamF12 medium) to a total volume of 800 µl to prepare solution A. Ham F12 medium was added to 96 µl of Lipofect AMINE reagent (manufactured by GIBCO BRL Inc.) to a total volume of 800 µl to prepare solution B. The solution A and the solution B were mixed, and the mixture was incubated at room temperature for 30 minutes and supplemented with 6.4 ml of HamF12 medium to prepare DNA-Lipofect AMINE mixed solution. 1.2×10^6 CHO DXB11 cells which had been inoculated in petri dish of 10 cm diam. (manufactured by Corning) on the previous day were washed with HamF12 medium, and supplemented with 8 ml of the DNA-Lipofect AMINE mixed solution. The cells were incubated in the presence of 5% CO_2/95% air at 37°C for 7.5 hours, the DNA-Lipofect AMINE mixed solution was removed and substituted with HamF12 medium supplemented with 10% inactivated bovine fetal serum (manufactured by JRH BIOSCIENCES Inc.), and the culture was continued for another 24 hours. The medium was again substituted with HamF12 medium supplemented with 10% inactivated bovine fetal serum, and the cultivation was continued for 24 hours. The cells were reinoculated at 10^3, 10^4, and 10^5 cells/10 ml HamF12 medium supplemented with 10% inactivated bovine fetal serum/petri dish of 10 cm diam. 2 days after the reinoculation, the medium was substituted with minimum essential medium αmedium without ribonucleotides and deoxyribonucleotides (manufactured by GIBCO

BRL, hereinafter abbreviated as MEMα(-) supplemented with 10% inactivated and dialyzed bovine fetal serum to initiate the selection of the cells having DHFR gene incorporated therein. The medium was substituted at every 3 or 4 days with new MEMα(-) supplemented with 10% inactivated and dialyzed bovine fetal serum, and colonies of DHFR positive cells started to form in about 2 weeks. The DHFR positive cells expressing shFas(nd29)-Fc were then cloned in accordance with the method of Nobuhara et al. (Jikken Igaku (Experimental Medicine), vol. 5, No. 11, 1987, pages 1108 - 1112). More illustratively, about 100 single colonies were cloned by using penicillin cup, and subcloned on 48 well plates (manufactured by NUNC). The cells were incubated by substituting the medium at every 3 or 4 days with new MEMα(-) supplemented with 10% inactivated and dialyzed bovine fetal serum, and the cultivation was continued at a greater scale when the well became confluent. When the cells proliferated to the extent that the wells of 6 well plates (manufactured by NUNC) were confluent, cell number was adjusted by inoculating at 2.5×10^5 cells/500 µl MEMα(-)supplemented with 10% inactivated and dialyzed bovine fetal serum/24 well plate (manufactured by NUNC), and the culture supernatant was harvested after 2 to 3 days. The content of shFas(nd29)-Fc in the supernatant was measured by EIA to select the cell line of high expression (CHO(pM1304)72).